11-13-06

Attorney's Docket No.: 18202-033US1 / 1051US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Johan Auwerx et al.	Art Unit	: 1636
Patent No.	: 7,098,025	Examiner	: Maria Marvich
Issue Date	: August 29, 2006	Conf. No.	: 6461
Serial No.	: 09/463,542	Cust. No.	: 20985
Filed	: December 11, 2002		
Title	: HUMAN PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA (PPARγ) GENE REGULATORY SEQUENCES AND USES THEREOF		

Attn: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL LETTER

Dear Sir:

Transmitted herewith are a Request for a Certificate of Correction pursuant to C.F.R. § 1.322 & 1.323 (3 pages), Certificate of Correction Form PTO-1050 (1 page), a copy of the 1st page of four journal articles originally mailed with the Information Disclosure Statement on June 16, 2005 (4 pages), and a return postcard for filing in connection with the above-identified application. One or more of the errors sought to be corrected were made by applicant, and a check for $100 is enclosed to cover the required fee of 37 CFR §1.20(a).

☒ The Commissioner is hereby authorized to charge any fees that may be due in connection with this paper or with this application during its entire pendency to Deposit Account No. 06-1050. A duplicate of this sheet is enclosed.

Respectfully submitted,

Stephanie Seidman
Reg. No. 33,779

Attorney Docket No. 18202-033US1 /1051US
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CERTIFICATE OF MAILING BY "EXPRESS MAIL"
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I hereby certify that this paper is being deposited with the United States Postal "Express Mail Post Office to Addressee" Service under 37 CFR §1.10 on the date indicated above and is addressed to: Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA, 22313-1450.

Stephanie Seidman

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION

Dear Sir:

Pursuant to 37 C.F.R. § 1.322 and 1.323, the patentee respectfully requests that a Certificate of Correction be issued for the above referenced patent to correct the following errors:

IN THE TITLE PAGES:

In Item [56] References Cited, in OTHER PUBLICATIONS:

in the second Auwerx et al., please insert —apo— between "LP1 and" and "C-III";

in Belluzzi et al., please replace "Belluzi" with —Belluzzi—, please replace "preparationon" with —preparation—, and please replace "crohn's" with —Crohn's—;

in Christy et al., please replace "preaddipocytes" with —preadipocytes—;

in Desvergene, B. and W. Wahli, please replace "*Expressio*" with —*Expression*—;

in Hertz et al., please replace " malic' " with —'malic'—;

in Ricote et al., please replace "lipoprotien" with —lipoprotein—, and please replace "(1988)" with —(1998)—;

in the fourth Schoonjans et al., please replace "byfibric-acid" with —by fibric-acid—;

in the fourth Tontonoz et al., please replace "mPPARγ2" with —mPPARγ2—; and

in the second Kliewer et al., please replace "J," with —J_2—.

CERTIFICATE OF MAILING BY "EXPRESS MAIL"
"Express Mail" Mailing Label Number **EV 740125847 US**
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Stephanie Seidman

NOV 28 2006

REMARKS

A Certificate of Correction (Form PTO-1050) incorporating the above changes is included with this Request. Since not all the errors are those of the Patent Office, a check is enclosed to cover the required fee. If it is determined that the fee amount is incorrect or if the check is missing, the Office is hereby authorized to charge the fee to Deposit Account No. 06-1050.

This Certificate of Correction seeks to correct obvious spelling, formatting, and typographical errors in the "OTHER PUBLICATIONS" section of the References Cited, Item [56]. The corrections in Belluzzi et al. seeks to correct obvious spelling errors by replacing "Belluzi" with —Belluzzi— and by replacing "preparationon" with —preparation—. Belluzzi et al. is also corrected by capitalizing the first letter of a proper name, thus replacing "crohn's" with —Crohn's—. The basis for the corrections to Belluzzi et al. are found on the first page of the Belluzzi journal article, a copy of which was previously provided with the Information Disclosure Statement mailed on June 16, 2005 and is attached herewith as evidence.

The correction in Desvergene, B. and W. Wahli seeks to correct an obvious spelling mistake by replacing "*Expressio*" with —*Expression*—. The correction in Hertz et al. seeks to replace " malic' " with —'malic'—. The basis for this correction is found on the first page of the Hertz journal article, a copy of which was previously provided with the Information Disclosure Statement mailed on June 16, 2005 and is attached herewith as evidence.

The correction in Ricote et al. seeks to correct an obvious spelling error by replacing "lipoprotien" with —lipoprotein— and also seeks to correct a typographical error by replacing "(1988)" with —(1998)—. The basis for the these corrections is found on the first page of the Ricote journal article, a copy of which was previously provided with the Information Disclosure Statement mailed on June 16, 2005 and is attached herewith as evidence.

The correction in the fourth Schoonjans et al. seeks to correct an obvious typographical error by replacing "byfibric-acid" with —by fibric-acid— such that the words "by" and "fibric-acid" are separated by a space. The basis for the this correction is found on the first page of the Schoonjans journal article, a copy of which was previously provided with the Information Disclosure Statement mailed on June 16, 2005 and is attached herewith as evidence.

Accordingly, none of the requested changes constitute new matter. Patentee respectfully requests correction of errors by issuance of a Certificate of Correction.

Respectfully submitted,

Stephanie Seidman
Reg. No. 33,779

Attorney Docket No. 18202-033US1 / 1051US
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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 7,098,025

APPLICATION NO : 09/463,542

DATED : AUGUST 29, 2006

INVENTOR(S) : AUWERX ET AL.

It is certified that an error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

IN THE TITLE PAGES:

In Item [56] References Cited, in OTHER PUBLICATIONS:

in the second Auwerx et al., please insert —apo— between "LP1 and" and "C-III"

in Belluzzi et al., please replace "Belluzi" with —Belluzzi—,
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in the fourth Tontonoz et al., please replace "mPPArγ2" with —mPPARγ2—

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The New England
Journal of Medicine

Volume 334 JUNE 13, 1996 Number 24

EFFECT OF AN ENTERIC-COATED FISH-OIL PREPARATION ON RELAPSES IN CROHN'S DISEASE

ANDREA BELLUZZI, M.D., CORRADO BRIGNOLA, M.D., MASSIMO CAMPIERI, M.D., ANGELO PERA, M.D., STEFANO BOSCHI, M.S., AND MARIO MIGLIOLI, M.D.

Abstract *Background.* Patients with Crohn's disease may have periods of remission, interrupted by relapses. Because fish oil has antiinflammatory actions, it could reduce the frequency of relapses, but it is often poorly tolerated because of its unpleasant taste and gastrointestinal side effects.

Methods. We performed a one-year, double-blind, placebo-controlled study to investigate the effects of a new fish-oil preparation in the maintenance of remission in 78 patients with Crohn's disease who had a high risk of relapse. The patients received either nine fish-oil capsules containing a total of 2.7 g of n−3 fatty acids or nine placebo capsules daily. A special coating protected the capsules against gastric acidity for at least 30 minutes.

Results. Among the 39 patients in the fish-oil group, 11 (28 percent) had relapses, 4 dropped out because of diarrhea, and 1 withdrew for other reasons. In contrast, among the 39 patients in the placebo group, 27 (69 percent) had relapses, 1 dropped out because of diarrhea, and 1 withdrew for other reasons (difference in relapse rate, 41 percentage points; 95 percent confidence interval, 21 to 61; $P<0.001$). After one year, 23 patients (59 percent) in the fish-oil group remained in remission, as compared with 10 (26 percent) in the placebo group ($P=0.003$). Logistic-regression analysis indicated that only fish oil and not sex, age, previous surgery, duration of disease, or smoking status affected the likelihood of relapse (odds ratio for the placebo group as compared with the fish-oil group, 4.2; 95 percent confidence interval, 1.6 to 10.7).

Conclusions. In patients with Crohn's disease in remission, a novel enteric-coated fish-oil preparation is effective in reducing the rate of relapse. (N Engl J Med 1996; 334:1557-60.)

CROHN'S disease is characterized by remission and relapse. The relapses are most likely to occur soon after patients enter remission and are more frequent in those with abnormalities in serum concentrations of acute-phase proteins.[1-3] Because fish oil has antiinflammatory actions, its use has been proposed in patients with several inflammatory diseases, including inflammatory bowel disease.[4-8] However, its unpleasant taste and its side effects, which include flatulence, heartburn, halitosis, belching, and diarrhea, make it unacceptable to many patients.[9-11]

We have found that the rate of absorption of the component n−3 fatty acids in fish oil is high when they are administered in the form of a new, enteric-coated preparation, so that the dose needed to achieve the incorporation of fish-oil fatty acids into phospholipid membranes is one third of that used previously.[12] As a result, the frequency of side effects is reduced, compliance increases, and long-term treatment becomes feasible for many patients.

In this study, we investigated the effects of the new, enteric-coated fish-oil preparation in the maintenance of remission in patients with Crohn's disease.

METHODS

Between May 1992 and September 1993, patients treated in our outpatient clinic who had an established diagnosis of Crohn's disease and were in clinical remission were evaluated for eligibility for this study with use of the Crohn's Disease Activity Index.[13] This index incorporates eight items — the daily number of liquid or very soft stools, abdominal pain, general well-being, extraintestinal manifestations of Crohn's disease, use of opiates to treat diarrhea, abdominal mass, hematocrit, and body weight — to yield a composite score ranging from 0 to 600. Higher scores indicate more disease activity. Patients with scores of 150 or less are considered to have inactive disease. The criterion for eligibility for our study was a score that had been below 150 for at least three months but less than two years.

In addition to having a score below 150 on this index, the patients had to have at least one of the following: a serum α_1-acid glycoprotein concentration above 130 mg per deciliter (normal reference range, <120 mg per deciliter), a serum α_2-globulin concentration above 0.9 g per deciliter (normal reference range, <0.8 g per deciliter), or an erythrocyte sedimentation rate of more than 40 mm per hour (normal reference range, <20 mm per hour). Patients were excluded if they were less than 18 or more than 75 years old, had received mesalamine, sulfasalazine, or corticosteroids in the previous

From the Institute of Clinical Medicine and Gastroenterology (A.B., C.B., M.C., M.M.) and the Department of Clinical Pharmacology (S.B.), University of Bologna, Bologna; and the Department of Gastroenterology, S. Giovanni Battista Hospital, Turin (A.P.) — both in Italy. Address reprint requests to Dr. Belluzzi at Via Vizzani, 36, 40138 Bologna, Italy.

Supported by Tillotts Pharma, Ziefen, Switzerland.

Thyromimetic mode of action of peroxisome proliferators: activation of 'malic' enzyme gene transcription

Rachel HERTZ*, Vera NIKODEM†, Alumit BEN-ISHAI*, Inna BERMAN* and Jacob BAR-TANA*‡

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Peroxisome proliferators induce thyroid-hormone-dependent liver activities, e.g. 'malic' enzyme, mitochondrial glycerol-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, S14 [Hertz, Aurbach, Hashimoto and Bar-Tana (1991) Biochem. J. **274**, 745–751]. Here we report that the thyromimetic effect of peroxisome proliferators with respect to 'malic' enzyme results from transcriptional activation of the 'malic' enzyme gene, mediated by binding of the peroxisome proliferator activated receptor (PPARα)/retinoid X receptor (RXRα) heterodimer to a 5'-flanking enhancer of the 'malic' enzyme promoter. The enhancer involved is distinct from the thyroid hormone response element of the 'malic' enzyme promoter and is partly homologous with that which mediates transcriptional activation of peroxisomal acyl-CoA oxidase by peroxisome proliferators. Hence transcriptional activation of thyroid-hormone-dependent liver genes by xenobiotic or endogenous amphipathic carboxylates collectively defined as peroxisome proliferators is mediated by a transduction pathway similar to that involved in transcriptional activation of peroxisomal β-oxidative genes and distinct from that which mediates thyroid hormone action.

INTRODUCTION

Amphipathic carboxylates known collectively as peroxisome proliferators (PPs) (e.g. fatty acids, xenobiotic aryloxyalkanoic acids, xenobiotic substituted dicarboxylic acids) induce liver peroxisomal β-oxidation as well as microsomal cytochrome *P*-4504-catalysed ω-oxidation of long-chain fatty acids (reviewed in [1]). Induction of these activities involves transcriptional activation of peroxisomal β-oxidative genes [2,3] and cytochrome *P*-4504 [4] mediated by binding of the peroxisome proliferator activated receptor (PPARα) [5] or the PPARα/retinoid X receptor (RXRα) heterodimer [6] to defined 5'-flanking enhancers of these genes [3,4,7].

In addition to regulating fatty acid β/ω-oxidation, PPs induce a calorigenic effect which, like that exerted by thyroid hormones [8–10], is characterized by a substantial reduction in liver phosphate and redox potentials [11]. Furthermore xenobiotic PPs induce in euthyroid and hypothyroid rats, or in rat hepatocytes cultured in thyroid-hormone-free media, liver activities classically considered to be thyroid-hormone-dependent, e.g. 'malic' enzyme, mitochondrial glycerol-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase and S14 [12,13]. The hepatic thyromimetic activity of PPs is not mediated by modulating plasma thyroid hormone levels or by PP binding to the thyroid hormone nuclear receptor [11,12]. Thus thyroid hormone binding to isolated liver nuclei or nuclear extract was competitively displaced by some but not all PPs capable of inducing the above liver activities [12]. Furthermore, in contrast with the thyromimetic effect induced by PPs in liver cells, no PP-induced increase in growth hormone was observed in cultured GH1 pituitary cells [12] and no increases in heart 'malic' enzyme or mitochondrial glycerophosphate dehydrogenase were observed in PP-treated animals under conditions where thyroid hormones were active [11], thus leaving open the question concerning the transduction pathway mediating the liver-specific thyromimetic effect induced by PPs.

To elucidate further the mode of action of PPs as inducers of liver thyroid-hormone-dependent genes, the induction of 'malic' enzyme by these agents has now been further analysed in order to define the elements of the transduction pathway that mediate the liver thyromimetic activity of PPs. Liver 'malic' enzyme was chosen as a model system because its established response to thyroid hormone is well characterized in terms of the ligand, receptor and response element involved [14–18].

MATERIALS AND METHODS

Animals

Euthyroid male albino rats weighing 150–200 g were fed with laboratory powdered chow diet containing the PPs specified. Food intake remained unaffected by the PPs added to the chow diet as specified. For hormonal induction, animals were injected intraperitoneally with 15 μg of tri-iodothyronine (T_3)/100 g body weight per day [12]. Animal studies were conducted in accord with humane animal care principles and procedures.

Cultured cells

Primary rat hepatocytes were isolated as previously described [12] and cultured on 60 mm Falcon culture dishes in RPMI 1640 medium containing 10% charcoal-treated fetal calf serum, 100 m-units/ml insulin, 10 μg/ml cortisol, 50 μg/ml streptomycin sulphate and 50 μg/ml penicillin G. CV-1 and COS-7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Gibco).

'Malic' enzyme activity

Liver homogenate was prepared in 4 vol. of 0.25 M sucrose. 'Malic' enzyme activity (EC 1.1.1.40) was assayed in the 100000 *g* cytosolic fraction as previously described [12].

Abbreviations used: AOX, acyl-CoA oxidase; CAT, chloramphenicol acetyltransferase; PP, peroxisome proliferator; PPARα, peroxisome proliferator activated receptor α; PPRE, peroxisome proliferator response element; RXRα, retinoid X receptor α; T_3R, thyroid hormone receptor; T_3, tri-iodothyronine; TRE, thyroid hormone response element; DMEM, Dulbecco's modified Eagle's medium; DTT, dithiothreitol.

‡ To whom correspondence should be addressed.

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 7614–7619, June 1998
Medical Sciences

Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein

MERCEDES RICOTE*, JANNET HUANG†, LUIS FAJAS‡, ANDREW LI§, JOHN WELCH*, JAMILA NAJIB‡, JOSEPH L. WITZTUM†, JOHAN AUWERX‡¶, WULF PALINSKI†¶, AND CHRISTOPHER K. GLASS*†¶‖

Divisions of *Cellular and Molecular Medicine, †Endocrinology and Metabolism, and §Cardiology, Department of Medicine, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0651; and ‡Unité 325, Institut National de la Santé et de la Recherche Médicale, Département d'Athérosclérose, Institut Pasteur, 59019 Lille, France

Communicated by Daniel Steinberg, University of California at San Diego, La Jolla, CA, April 23, 1998 (received for review March 27, 1998)

ABSTRACT **The peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-dependent transcription factor that has been demonstrated to regulate fat cell development and glucose homeostasis. PPARγ is also expressed in a subset of macrophages and negatively regulates the expression of several proinflammatory genes in response to natural and synthetic ligands. We here demonstrate that PPARγ is expressed in macrophage foam cells of human atherosclerotic lesions, in a pattern that is highly correlated with that of oxidation-specific epitopes. Oxidized low density lipoprotein (oxLDL) and macrophage colony-stimulating factor, which are known to be present in atherosclerotic lesions, stimulated PPARγ expression in primary macrophages and monocytic cell lines. PPARγ mRNA expression was also induced in primary macrophages and THP-1 monocytic leukemia cells by the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA). Inhibition of protein kinase C blocked the induction of PPARγ expression by TPA, but not by oxLDL, suggesting that more than one signaling pathway regulates PPARγ expression in macrophages. TPA induced the expression of PPARγ in RAW 264.7 macrophages by increasing transcription from the PPARγ1 and PPARγ3 promoters. In concert, these observations provide insights into the regulation of PPARγ expression in activated macrophages and raise the possibility that PPARγ ligands may influence the progression of atherosclerosis.**

The peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear receptor superfamily of transcription factors that regulate patterns of gene expression in response to the binding of small molecular weight ligands (1–3). PPARγ mRNA is most highly expressed in adipose tissue, the adrenal gland, spleen, and large colon (4–7). Several lines of evidence indicate that PPARγ plays an important role in regulating adipocyte differentiation and glucose homeostasis. PPARγ and the retinoid X receptor (RXR) form heterodimers on regulatory elements in a number of adipose-specific promoters that stimulate transcription in response to PPARγ or RXR-specific ligands (3, 5, 8, 9). Furthermore, forced expression of PPARγ in certain fibroblast cell lines induces adipocyte differentiation in a manner that is strongly potentiated by PPARγ- and RXR-specific ligands (8–11). Although the identities of the ligands that regulate PPARγ activity *in vivo* remain to be established with certainty, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15ΔPGJ$_2$) and certain polyunsaturated fatty acids have been demonstrated to stimulate PPARγ-dependent transcription (9, 10, 12, 13). In addition, synthetic ligands such as troglitazone and BRL49653 have been identified that are specific PPARγ activators (14). Troglitazone and structurally related thiazolidinediones significantly reduce peripheral resistance to insulin in obesity and type 2 diabetes mellitus in both animals and humans and have recently been instituted as adjunctive therapy in diabetic patients (15–18).

The roles of PPARγ in other tissues are poorly understood. Recent studies indicate that PPARγ is expressed in cells of the monocyte/macrophage lineage (19–21). Several lines of evidence suggest that PPARγ may exert anti-inflammatory effects by negatively regulating the expression of pro-inflammatory genes. Treatment of peritoneal macrophages with 15ΔPGJ$_2$ or several synthetic PPARγ ligands reduced the expression of inducible nitric oxide synthase by interferon γ (IFN-γ) and inhibited induction of gelatinase B and scavenger receptor A gene transcription in response to phorbol ester stimulation (20). Similarly, treatment of primary human monocytes with PPARγ-specific ligands blocked phorbol ester induction of interleukin 6 (IL-6), tumor necrosis factor α, and IL-1β (21). Anti-inflammatory effects of PPARγ ligands have not as yet been established *in vivo*, however, and it is possible that PPARγ exerts complex effects on macrophage function that are not strictly related to inflammation.

Macrophages are thought to play critical pathogenic roles in several chronic inflammatory diseases, including atherosclerosis (reviewed in refs. 22 and 23). Fatty streaks, the earliest visible lesions of atherosclerosis, contain large numbers of macrophage foam cells derived from circulating monocytes that adhere to activated endothelium and migrate into the artery wall (reviewed in ref. 24). These cells subsequently differentiate into macrophages that express the scavenger receptor A gene, as well as other scavenger receptors that mediate the uptake of oxidized low density lipoprotein (oxLDL) (25). Because these receptors are not subject to negative regulation by high levels of intracellular cholesterol, massive accumulation of cholesterol esters can occur in macrophages, resulting in foam cell formation. In addition to their uptake of oxLDL, macrophage foam cells are thought to influence the progression of atherosclerosis by several additional mechanisms, including promoting LDL oxidation (24), secretion of pro-inflammatory cytokines and other humoral factors that exert paracrine and autocrine effects in the artery wall (22, 23), and secretion of matrix metalloproteinases that have been suggested to remodel extracellular matrix proteins in arterial

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Abbreviations: PPARγ, peroxisome proliferator-activated receptor γ; oxLDL, oxidized low density lipoprotein; 15ΔPGJ$_2$, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; M-CSF, macrophage colony-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; IFN-γ, interferon γ; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; LPS, lipopolysaccharide; PKC, protein kinase C.

¶The Auwerx, Palinski, and Glass laboratories made equivalent contributions to these studies.

‖To whom reprint requests should be addressed. e-mail: cglass@ucsd.edu.

Eur. J. Biochem. 216, 615–622 (1993)

780

yl-CoA synthetase mRNA expression is controlled by fibric-acid derivatives, feeding and liver proliferation

Kristina SCHOONJANS[1], Bart STAELS[1], Paul GRIMALDI[2] and Johan AUWERX[1]

[1] Laboratoire de Biologie des Régulations chez les Eucaryotes, Centre de Biochimie, Université de Nice-Sophia Antipolis, France
[2] Laboratoire de Biologie du Développement du Tissu Adipeux, Centre de Biochimie, Université de Nice-Sophia Antipolis, France

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Several enzymes of the β-oxidation pathway have been shown to be induced after stimulation with peroxisomal proliferators, including several hypolipidemic drugs. We investigated the regulation of the long-chain-acyl-CoA synthetase (ACS) gene in the liver. Fenofibrate, a hypolipidemic drug and potent peroxisomal proliferator, induced ACS gene expression in several tissues. In liver, large increases in ACS mRNA levels and ACS activity were observed after fenofibrate administration. Adipose tissue ACS mRNA levels and ACS activity were also stimulated upon fibrate treatment but to a lesser extent in comparison with liver ACS mRNA. Kidney ACS mRNA was only weakly induced, except for the highest dose and the longest treatment period, where a strong induction was observed. In contrast to these tissues, heart ACS mRNA and ACS activity remained almost unchanged after fenofibrate treatment. These effects of fenofibrate could be reproduced by other fibrates such as clofibrate. In addition, it is demonstrated that both nutritional composition and liver proliferation trigger ACS gene expression in liver. Consequently, these data suggest that ACS is a highly regulated enzyme with a potentially important control function in lipid metabolism.

Fibric-acid derivatives belong to a class of hypolipidemic drugs, which are used to lower plasma triacylglyceride levels in diet-resistant hyperlipidemic patients (Brown, 1987; Grundy and Vega, 1987; Sirtori and Franceschini, 1988; Balfour et al., 1990). Besides this pharmacological effect in man, fibrates are known to be potent peroxisomal proliferators in rodents. Together with a group of apparently unrelated compounds (e.g. herbicides, phthalate and adipate-ester plasticizers), fibrates are able to activate the β-oxidation system of fatty acids in the liver. It has been demonstrated that fibrates induce a large number of enzymes implicated in the β-oxidative pathway including acyl-CoA oxidase (ACO; Chatterjee et al., 1983; Osumi et al., 1984), enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (multifunctional enzyme; Reddy et al., 1986; McQuaid et al., 1987; Chatterjee et al., 1987) and thiolase (Hijikata et al., 1987). The induction of these enzymes leads to increased β oxidation, peroxisomal proliferation, liver hypertrophy and hyperplasia. Ultimately, hepatocarcinoma can develop in animals treated for a prolonged period with fibrates (Reddy et al., 1980). Recently, it was proposed that peroxisome proliferators, such as fibrates, might induce the activity of the genes for these enzymes by activation of a group of transcription factors, the peroxisomal-proliferator-activated receptors (PPAR; Isseman and Green, 1990; Dreyer et al., 1992; Gottlicher et al., 1992; Schmidt et al., 1992). PPAR belong to the steroid-hormone-receptor family. The actual ligands for PPAR are unknown at present, but it has been shown that fatty acids and fibrates can activate them (Gebel et al., 1992; Gottlicher et al., 1992). Activated PPAR allow the expression of genes with a PPAR responsive element (RE) in their 5′ upstream regulatory sequences (URS). Several key enzymes of the β-oxidation pathway, which are induced after administation of peroxisomal proliferators, have been shown to contain a PPAR-RE in their 5′ URS. The gene expression of ACO, the rate-limiting enzyme in peroxisomal β-oxidation and widely used as a marker of peroxisomal proliferation, has been shown to be regulated by the interaction of PPAR with a PPAR-RE in the 5′ URS (Osumi et al., 1991; Tugwood et al., 1992; Dreyer et al., 1992). Transactivation by PPAR, through interaction with its responsive element, has recently also been identified for another enzyme implicated in β-oxidation, i.e. enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (Zhang et al., 1992; Bardon et al., 1993).

At present, the enzyme which initiates the β-oxidation pathway, however, has not been studied in relation to peroxisomal-proliferator-induced activation. This enzyme, ATP-dependent long-chain-acyl-CoA synthetase (ACS), is a pivotal enzyme for lipid metabolism (Kornberg and Pricer, 1953; Garland et al., 1970; Suzuki et al., 1990; Yamamoto et al., 1990). ACS catalyses the first step of fatty-acid metabolism, i.e. the conversion of fatty acids to acyl-CoA derivatives (Suzuki et al., 1990; Yamamoto et al., 1990). These intermediates are mainly used for synthesis of triacylglycerols and β-oxidation. Other important roles of acyl CoA in lipid metabolism are desaturation and chain elongation of fatty acids, inhibition of acetyl-CoA carboxylase (Numa and Tanable, 1984) and esterification with cholesterol. In addition, acyl-

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Abbreviations. ACO, acyl-CoA oxidase; ACS, long-chain-acyl-synthetase; PPAR, peroxisomal-proliferator-activated recep-; RE, responsive element; URS, upstream regulatory sequence.
Enzyme. Acyl-CoA synthetase (EC 6.2.1.3).